Interaction of metal ions with 5'-guanosine monophosphate—Evidence for binding through N(1) in solution

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Received 24 November 1989; revised 5 March 1990; accepted 16 April 1990

The stability constants of Cu(II), Ni(II), Zn(II) and Co(II) binary complexes (1:1) with 5-guanosine monophosphate (5-GMP) as the primary ligand and of the corresponding ternary complexes (1:1:1) with glycine, histidine and histamine as the secondary ligands have been determined potentiometrically at 35°C and 0.10 M (KNO₃) ionic strength. In the acidic medium, N (7) and phosphate oxygen of 5'-GMP are found to be more favoured coordination sites; the preference for N(1) increases with increase in pH. This multi-site bonding behaviour of 5'-GMP towards cations in solution has been investigated and the influence of pH on the coordinating abilities of 5'-GMP assessed. The influence of secondary ligands on the stabilities of M(II)-5'-GMP systems is also discussed. Species distribution curves have been obtained using the computer programme BEST.

Metal ion interactions with ligands having different donor atoms provide an opportunity to study their contributions to the stabilities of metal complexes. This type of investigations become much more relevant when the metal ion interactions with ligands of biological importance are considered. We have been interested for some time in these interactions, in particular with nucleosides and nucleotides, because of their multi-site binding behaviour towards cations in solution. These have been several attempts both in solution and solid state to probe this behaviour of nucleic acid components. The present study is yet another attempt in this direction.

Earlier work in solution on 5'-guanosine monophosphate (5'-GMP) relates to the interaction of Cu(II) at pH 7. ¹³C and ³¹P NMR studies of 5'-GMP with Mn²⁺ indicated the presence of multiple binding sites. Earlier studies have shown that the metals are bonded to 5'-guanosine monophosphate (5'-GMP, structure I) through N (7) and the oxygen atom of H₂O molecules (which are hydrogen bonded to phosphate group). Aoki et al. have shown that Cu(II) is directly bonded to N (7) of the base and to the phosphate moiety in [Cu(5'-GMP)]₉(H₂O)₉ compound. Pt(II) and Mg(II) were also found to be N (7)-bonded with 5-GMP. However, only Mg(II) was found to be directly coordinated to phosphate at lower pH.

Recently Perno et al. have studied the interaction of Ni(II) and cis-[NH₃]₂Pt²⁺ with deoxy-guanosine monophosphate.

However, there are discrepancies between the results obtained in solution and solid states. The major reason for this seems to be the high dependence of metal binding sites on pH. Obviously, the experimental pH has to be monitored very carefully in order to get reliable information.

In addition to the ambiguity due to base versus phosphate binding, purine nucleotides display a dichotomy between binding through N (1) and N (7). In 5'-GMP, N (7) is deprotonated in slightly acidic solutions and therefore metal binds preferentially through N (7). In neutral and basic solutions metal ions bind through N (1).

Though the NMR spectroscopy has proved to be one of the most powerful techniques for determining the binding sites of the metal ions to nucleotides, the rapid exchange of acidic protons is a problem and many of these investigations have been made in solvents such as DMSO. In general these solutions are very different from the aqueous ones. Similarly, crystal structure determinations have been confined mainly to samples grown in acidic solutions where N (1) is protonated. In view of this it was thought important to investigate the metal ion interactions with 5'-GMP in aqueous solution covering the pH range 3-10.

In the present paper interactions of Cu(II), Ni(II), Zn(II) and Co(II) with 5'-guanosine monophosphate (binary and ternary systems) have been
studied to get further information on the metal binding sites in absence and presence of biologically important secondary ligands. The secondary ligands used in the ternary systems are glycine, histidine and histamine. The structures of primary and secondary ligands showing the dissociation steps are depicted in Charts I and II.

**Materials and Methods**

5'-Guanosine monophosphate (5'-GMP) was obtained from Fluka (Switzerland), and glycine (gly), histidine (histi) and histamine (hista) were obtained from Sigma chemical Company (USA).

The experimental method consisted of a potentiometric titration of solutions containing metal ion, nucleotide and secondary ligands in (1:1) and (1:1:1) ratios at 35±0.1°C with standard NaOH solution. The ionic strength was maintained constant by using 0.1M (KNO₃) as the supporting electrolyte and relatively low concentration of the ligand and metal ion (1×10⁻³ M). Other experimental details can be found elsewhere.

**Calculations**

**Binary systems**

In M(II)-5'-GMP (1:1) binary systems two types of interactions have been observed. Cu(II) and Zn(II) form one type of complexes and the rest of the metal ions another type.

In the case of Cu(II)- and Zn(II)-5'-GMP systems, the following equation was used in the buffer region m = 1-3.

\[ M + H₂L⁻ \rightleftharpoons ML + 2H⁺ \]  

For Ni(II) and Co(II), the following equations were employed. In the buffer region m = 1-2, Eq. 2 was used,

\[ M + H₂L⁻ \rightleftharpoons MHL + H⁺ \]  

and in the buffer region m = 2-3, Eq. 3 was used.

\[ MHL \rightleftharpoons ML + H⁺ \]  

**Ternary systems**

**M(II)-5'GMP-gly (1:1:1) system**

Eqs 4 and 5 were used to calculate stability constants for Cu(II).

\[ M + H₃L + H₂A \rightleftharpoons M(H₃L)A + 3H⁺ \]  

For the rest of the metal ions, equations 6 and 7 were used. In the buffer region m = 0-2, Eq. 6 was used,

\[ M + H₃L + H₂A \rightleftharpoons M(H₃L)HA + 2H⁺ \]  

and in the buffer region m = 2-5, Eq. 7 was used,

\[ M(H₃L)HA \rightleftharpoons MLA + 3H⁺ \]  

**M(II)-5'GMP-histi/hista (1:1:1) systems**

Equations 4 and 5 were employed to calculate the stability constants of Cu(II) complexes of 5'-GMP with histidine and histamine as secondary ligands.

For Ni(II), Zn(II) and Co(II), Eqs 8 and 9 were used; in the buffer region m = 1-4, Eq. 8 was used,

\[ M(H₂L)H₂A \rightleftharpoons M(HL)A + 3H⁺ \]  

while Eq. 9 was used between m = 4 and 5.
\[ M(ML)A = MLA + H^+ \quad \ldots (9) \]

It should be noted here that in the case of histidine and histamine systems [except for Cu(II)], the buffer region \( m = 0-1 \) was not taken into account for the calculation of constants as the titration curves overlapped with the free ligand curve.

All calculations were performed using the experimental data based on the trends in the titration curves on a CASIO-PB-100 personal computer with the aid of suitable material balanced equations. The species distribution curves for various systems were obtained with the help of the computer programme BEST\textsuperscript{13}.

Results and Discussion

Binary \( M(\text{II})-5'-\text{GMP} \) system

The titration curve of Cu(II)-5'-GMP system (Fig. 1e) showed an inflection at \( m = 1 \) followed by a buffer region. Accordingly, it was assumed that a normal (1:1) complex was formed in the buffer region \( m = 1-3 \). The constant \( K_{ML}^M \) was calculated using Eq. (1). The behaviour of Zn(II) was similar to that of Cu(II).

The titration curve of Co(II) (Fig. 1d) showed inflections at \( m = 1 \) and \( m = 2 \); the corresponding protonated \( (K_{ML}^M) \) and normal \( (K_{ML}^{MHL}) \) (1:1) constants were calculated in the above buffer region using Eqs 2 and 3. Similar trends were obtained in the case of Ni(II).

For all the above metal ions no attempt was made to calculate the constants in the buffer region \( m = 0-1 \) as the titration curves in this region overlapped with the free ligand curve. All the above binary constants are presented in Table 1.

Ternary (1:1:1) systems

Glycine system

The mixed ligand titration curve of Cu(II)-5'-GMP-gly (Fig. 1c) in a 1:1:1 ratio showed an inflection at \( m = 3 \) followed by a buffer region. Formation of protonated and normal (1:1:1) complexes was assumed in the buffer regions before and after \( m = 3 \) respectively. The constants \( K_{M(H,L)A}^M \) and \( K_{MAL}^{MHL} \) were calculated using Eqs 4 and 5. For Ni(II), Zn(II) and Co(II) systems, an inflection was observed at \( m = 2 \) followed by a buffer region. The corresponding constants were calculated using equations 6 and 7.

The various constants pertaining to the above interactions are listed in Table 1.

Histidine/histamine system

The trends in the Cu(II)-5'-GMP-histi/hista titration...
interaction curves were found to be similar to those in Cu(II)-S'-GMP-gly system.

The mixed ligand titration curve of Co(II)-5'-GMP-histidine (Fig. 1b) or histamine showed inflections at \( m = 1 \) and \( m = 4 \). The corresponding constants were calculated using Eqs 8 and 9. Similar results were observed in the case of Ni(II) and Zn(II) also (Table 2).

The titration curve of free 5'-GMP showed (Fig. 1a) inflections at \( m = 1 \) and 2 followed by a buffer region indicating the stepwise dissociation of its protons. These are assigned to dissociations of N(7)-H, secondary phosphate hydrogen and N(1)-H respectively. The values of dissociation constants agreed well with the literature values for 5'-GMP\(^{14,15}\) as well as those for its nucleoside\(^{14} \) and base\(^{2} \) which further confirmed our assignments about proton dissociation.

The stability constants pertaining to the Cu(II), Zn(II), Ni(II) and Co(II)-5'-GMP interaction are presented in Table 1. No interaction was observed with Mg(II) and Ca(II). The stability constants of 5'-GMP complexes were higher than the corresponding constants of guanosine\(^{14} \) complexes. The higher stabilities in case of former can be attributed to the involvement of phosphate moiety in metal bonding in addition to the base coordination. It can be seen from Table 1 that Cu(II) and Zn(II) differ significantly in their interactions with 5'-GMP as compared to Ni(II) and Co(II). This type of selective interactions are an indication of the differences in the mode of bonding in these complexes. However, no interaction was observed for these metal ions up to \( pH \) 4.5. These results are not surprising when the \( pH \)-dependent coordinating ability of 5'-GMP is considered.

Among the three potential donor sites of 5'-GMP [N(7), phosphate oxygen and N(1)], N(7) and phosphate oxygen are found to be more favoured sites in acidic medium and the preference for N(1) increases as \( pH \) increases. Since no interaction was observed in acidic medium, the possibility of involvement of N(7) in coordination to metal can be ruled out. This leaves only secondary phosphate oxygen and N(1) as potential donor sites. The experimental \( pH \) range of the present study confirms this assumption which is further corroborated by the species distribution curves.

The species distribution curves for Ni(II)-5'-GMP indicate that the formation of protonated (1:1) complex starts at \( pH \sim 5.5 \) and reaches a maximum of 26% around \( pH \) 7.5. The predomi-
nant species in this system also seems to be the normal (1:1) complex which attains a maximum value of 68% at pH around 9. Based on these data it is proposed that in the protonated complex only phosphate oxygen is involved in metal binding and in the normal (1:1) complex, N(1) coordinates along with hydrogen bonded phosphate oxygen.

The ternary constants of various systems are listed in Tables 1 and 2. It may be seen from the tables that in these systems also different types of complexes are formed.

The stability constants for the protonated ternary Cu(II) system decrease in the order: histi > hista ≈ gly. This suggests that the action of histidine is neither glycine-like nor histamine-like; it acts as a terdentate ligand coordinating through all its available donor groups (i.e. carboxylate, imidazole and amino groups). This shows that in these systems only N(7) of 5'-GMP is involved in bonding in addition to donor sites of histamine and histidine. However, in the case of normal complexes the differences in the stabilities are narrowed down indicating that histidine acts as a bidentate ligand. This is not surprising because of the fact that in this buffer region (m = 3 to 5) two donor sites [phosphate oxygen and N(1)] from the 5’-GMP are involved in bonding. A species distribution curve for Cu(II)-5’-GMP-gly (1:1:1) system (Fig. 2) further confirms this assumption.

When the protonated ternary complexes of Ni(II) and Co(II) with histidine, histamine and 5’-GMP are compared, it is observed that histidine forms more stable complexes than histamine. This may be attributed again to the differences in their modes of bonding. In the case of Zn(II) the stability constants for both the systems are almost same. The reduced stability of Zn(II)-5’-GMP-histidine ternary complex may be due to the lower coordination number of Zn(II) where histidine may act as a bidentate ligand. However, in the case of normal complexes of Ni(II) and Co(II) the trend is reversed. In the buffer region m = 4-5, histidine may act as a bidentate ligand coordinating only through imidazole and amino nitrogens. It is likely that a hydrogen bonding type of interaction between COO⁻ of histidine and NH₂ of 5’-GMP takes place as a result of which the COO⁻ group is unable to coordinate to the metal. Such an interaction disfavour the formation of ternary complexes in solution due to the entropy factor as the ligands become more rigid as a consequence of bridge formation between two non coordinating groups and also due to the different degrees of solvent interaction with the metal ion. This may be the reason for the lower stabilities of histidine complexes compared to those of histamine complexes. Based on these conclusions, a tentative structure for Ni(II)-5’-GMP-histidine is proposed (Fig. 3).

Fig. 2—Species distribution curve for Cu(II)-5’-GMP-gly (1:1:1) ternary system

Fig. 3—Tentative structure for Ni(II)-5’-GMP-histidine complex (1:1:1) in solution
Finally, it is clear from the above investigations that the metal ion interactions with nucleic acid components are highly selective, in particular with mononucleotides where the presence of phosphate moiety adds a new dimension.

Acknowledgement
The financial assistance from UGC, New Delhi through Scheme No. (F-12-87/84 SR-III) is gratefully acknowledged.

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